2001

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Cell, Vol. 106, 255–258, August 10, 2001, Copyright ©2001 by Cell Press

Dendritic Cells: Specialized and Regulated Antigen Processing Machines

Minireview

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It is easy to be fascinated by dendritic cells (DCs), not only because of their pivotal role in the immune response, but also because of the elegance with which they perform their tasks. Although DCs comprise multiple subsets (Liu, 2001 [this issue of *Cell*]), all are unusually effective at antigen processing and presentation. DCs can take up a diverse array of antigens and present them to T cells as peptides bound to both MHC class I and II products. Relative to other antigen presenting cells, DCs are adept at stimulating naïve T cells. DCs also control the quality of the T cell response, driving naïve lymphocytes into distinct classes of effectors. These antigen-specific, adaptive responses are critical for resistance to infections and tumors. Conversely, DCs can also generate regulatory T cells that suppress activated T cells, a function of likely importance in autoimmunity and transplant rejection.

In addition to their role in adaptive responses, DCs play a critical role in innate immunity. In some respects, DCs are as active in responding to microbial challenge as DCs can produce copious amounts of cytokines involved in host defense, such as IL-12 and both type I and II interferons. DCs also activate NK and NKT cells, innate lymphocytes that rapidly kill select targets and produce important cytokines.

Initiating immunity may be only half of the story, however. It is becoming increasingly clear that DCs also capture antigens against which immunity is normally avoided. These include environmental proteins chronically found in the respiratory and digestive tracts (Vermaelen et al., 2001), as well as self antigens derived from tissues exhibiting constitutive cell turnover (Huang et al., 2000). Conceivably, the capture of proteins in the steady state, i.e., in the absence of microbial or other perturbations, allows DCs to control tolerance to self and "normal" environmental constituents.

How is it that DCs can mediate such diverse, almost contradictory functions in the immune response and do so with such efficiency? Here, we concentrate on newly appreciated specializations that enable DCs to capture and process antigens in a distinct manner relative to other antigen presenting cells. These qualitative and quantitative distinctions are regulated by inflammatory and microbial stimuli in a developmental process termed "maturation." In effect, maturation couples innate to adaptive responses and is potentially pivotal in the self-nonself distinction orchestrated by DCs.

Terminal Differentiation Determines Immunity

DCs in culture exist in two functionally and phenotypically distinct states, immature and mature (Figure 1). Immature cells are adept at endocytosis and express relatively low levels of surface MHC class I and II products and costimulatory molecules (e.g., CD86). Abundant MHC class II molecules are synthesized, but they are mainly sequestered intracellularly in late endocytic compartments (lysosomes; Figure 1, left). As in other MHC class II-expressing cells, the majority of new class II molecules are targeted directly to endosomes and lysosomes following their diversion from the secretory pathway upon exit from the trans-Golgi network (Pierre et al., 1997). However, a variable fraction of MHC class II is likely to reach lysosomes following endocytosis from the plasma membrane, particularly in monocyte-derived DCs (Cella et al., 1997).

Antigens can be avidly taken up by immature DCs and targeted to MHC class II-positive lysosomes. However, they are not efficiently utilized for the formation of MHC II-peptide complexes, but are retained for use as immunogenic peptides days later (Inaba et al., 2000; Turley et al., 2000). Immature cells do form SDS-stable class II dimers, but their presence does not correlate with the production of immunogenic complexes (Pierre et al., 1997). Thus, immature DCs in culture can take up antigen but do not present it efficiently to T cells. Most DCs in peripheral tissues in situ are of the immature phenotype, the prototype being Langerhans cells in the epidermis.

After detecting microbial products or proinflammatory cytokines, immature DCs transform into mature DCs, cells with a reduced capacity for antigen uptake but now with an exceptional capacity for T cell stimulation. This transition is accompanied by a dramatic cytoplasmic reorganization highlighted by a redistribution of MHC class II from intracellular compartments to the plasma membrane. Class II molecules appear to exit from the lysosomes, then to reside transiently in nonlysosomal cytoplasmic structures (class II vesicles or CIIV), and finally to accumulate on the cell surface (Figure 1, right). In tandem, surface costimulatory molecules (CD80, CD86), MHC class I, and T cell adhesion molecules (e.g., CD48 and CD58) are all upregulated. The maturing DCs also upregulate the capacity to generate functional peptide-MHC II complexes from newly internalized antigen or from antigen internalized prior to the maturation signal (Inaba et al., 2000; Turley et al., 2000). The cells extend long "dendritic" processes (actually, membrane folds) that may increase opportunities for T cell capture and interaction. DCs also remodel their profile of chemokine receptors that facilitate homing to lymphoid organs.

Much of what is known concerning DC maturation has been learned from DC cultures, either cells differentiated with GM-CSF and IL-4 from nongrowing human blood monocytes or from proliferating bone marrow-derived precursors (mouse, rat, or human). The current view has immature DCs encountering antigen in the periphery and carrying it to lymphoid organs, maturing en route. While valuable in general terms, this view is probably too simple for describing the DC system in vivo. For example, DCs migrating from the periphery may not always be the ones that present antigen in the lymph nodes. Rather, migrating DCs may transfer their captured antigens to other DCs for presentation. The trans-

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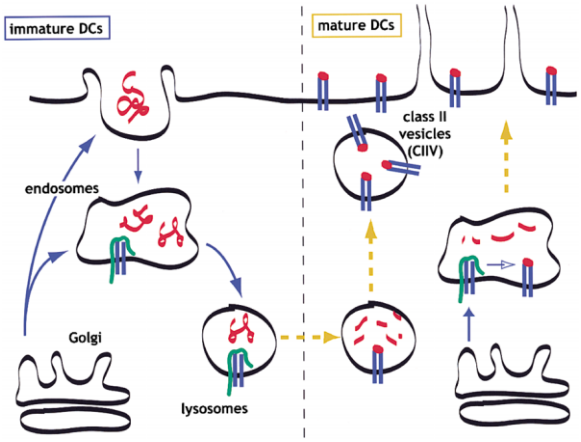

Figure 1. Dendritic Cell Maturation Induces Multiple Alterations in the Function and Intracellular Transport of MHC Class II Molecules

fer could occur either by phagocytosis of the antigen-loaded DCs (Inaba et al., 1998) or by the release of antigen-bearing vesicles (exosomes) derived from a DC's lysosomal compartment (Thery et al., 1999). Another oversimplification is the idea that all DCs within a lymph node are mature. Most DCs within lymph node in situ may be able to form MHC-peptide complexes, but they are otherwise immature and may function to induce peripheral tolerance (see below).

Signals Leading to Dendritic Cell Maturation

DC maturation is typically triggered by products of microbial or viral pathogens, such as LPS, CpG DNA, or dsRNA. For many or all of these, one or more members of the family of Toll-like receptors (TLRs) play a critical role (Kaisho and Akira, 2001). It remains unclear if bacterial products interact directly or indirectly with TLRs, and in some cases, endocytosis of the maturation stimulus may precede TLR activation. The involvement of TLRs in maturation provides an attractive mechanism by which DCs link innate to adaptive immunity.

Proinflammatory cytokines, such as TNFα and IL-1β also trigger maturation, as does ligation of CD40, a TNF receptor family member abundant on DCs. TLRs, IL-1R, and TNF receptors each lead to NF-κB activation, a hallmark of mature DCs. DC maturation also can be initiated by a variety of noninflammatory and pathogen-unrelated stimuli. In DC cultures, this is exemplified by the fact that gentle disruption of cell-cell contacts induces maturation (Pierre et al., 1997). We suspect that different stimuli are likely to trigger qualitatively different states of maturation, suggesting that either via TLRs or other receptors, DCs "decode" environmental signals, allowing for the development of mature DCs capable of polarizing T cell responses or inducing tolerance. It is already known, for example, that certain maturational stimuli (e.g., LPS) will favor the development of Th1 versus Th2 cells in vitro, in part due to the release of IL-12 by DCs that induce Th1 polarity.

Dendritic Cell Maturation and Endocytosis

Most immature DCs exhibit three types of endocytosis: macropinocytosis, phagocytosis, and clathrin-mediated endocytosis. In culture, the bulk of fluid uptake is thought to reflect macropinocytic activity. Correspondingly large amounts of plasma membrane (and receptor-bound ligands) must also be endocytosed. The internalized volumes of fluid are high, possibly explaining the expression of aquaporins 3 and 7 which provide for water efflux (de Baey and Lanzavecchia, 2000).

Macropinocytosis and phagocytosis are related processes, both depending on regulated actin assembly and thus the activity of the Rho family GTPases Cdc42 and Rac (Garrett et al., 2000; West et al., 2000). Phagocytosis is triggered by the attachment of extracellular particles to surface receptors, which in turn signal particle uptake. From an evolutionary perspective, bacterial or particle ingestion by phagocytosis is probably the most physiologically relevant form of antigen uptake by DCs. In fact, derivation of antigenic peptides from phagocytosed antigens is particularly efficient (Inaba et al., 1998).

The downregulation of endocytosis begins soon after the receipt of a maturation signal. Mechanistically, it may reflect a reduction in levels of active (GTP-bound) Cdc42, presumably via the regulation of a guanine nucleotide exchange factor (Garrett et al., 2000). Clathrin-coated vesicle formation appears to continue, so mature DCs must not be considered as being completely incapable of endocytosis.

Some DC subsets may exhibit novel strategies for endocytosis. Epidermal Langerhans cells, for example, reside in an environment not characterized by abundant extracellular fluid, making it unlikely that they survey their surroundings by macropinocytosis. An intriguing alternative is provided by Birbeck granules (BGs), cytoplasmic membrane-bound tubules exhibiting distinct internal striations. BGs are enriched in a lectin, langerin (CD207), whose expression even in fibroblasts can induce granule formation (Valladeau et al., 2000). It remains to be established whether langerin, or analogous lectins, are physiologically important for antigen uptake.

Mechanisms for the Upregulation of Peptide-MHC II Complexes During Maturation

One of the hallmarks of DC maturation is a dramatic increase in surface MHC class II and costimulatory molecules. MHC class II products can increase some 5- to 20-fold while CD86 increases up to 100-fold. The upregulation of surface MHC class II largely reflects posttranslational events. There is only a slight increase in the amount of MHC II mRNA following receipt of a maturation signal; in mature DCs, MHC class II synthesis actually decreases. Instead, there are major changes in the intracellular transport of MHC class II molecules.

In immature cells, as mentioned above, new MHC class II accumulates in late endosomes and lysosomes while in mature DCs, class II molecules accumulate at the cell surface (Cella et al., 1997; Pierre et al., 1997). The reasons for this change undoubtedly reflect the contributions of multiple factors, and may vary among individual MHC class II haplotypes and DC populations. One element of the mechanism, possibly unique to DCs, may involve the regulation of cathepsin S (cat S) activity by the specific antiprotease cystatin C (Pierre and Mellman, 1998). Cat S has a major role in the cleavage of the MHC II-associated invariant (Ii) chain. The lumenal aspect of Ii chain occupies the peptide binding groove of newly synthesized MHC II $\alpha\beta$ dimers, while the cytoplasmic domain contains a lysosomal targeting signal. In immature mouse DCs, levels of cystatin C appear sufficient to attenuate cat S activity, slowing Ii chain processing and favoring MHC II transport to lysosomes.

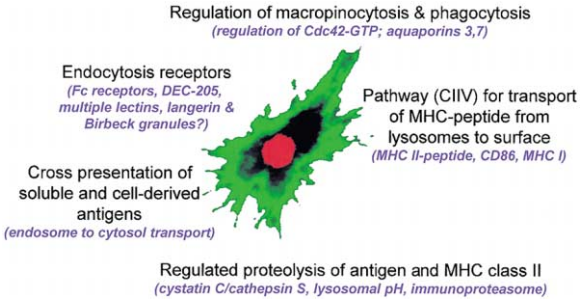

Figure 2. Dendritic Cell Specializations that Control the Formation of MHC-Peptide Complexes

After maturation, intracellular cystatin C decreases enhancing cat S activity and allowing a greater fraction of new $\alpha\beta$ dimers to avoid lysosomes and reach the cell surface. The downregulation of endocytosis must also contribute to the enhanced residence of MHC class II on the plasma membrane of mature DCs. Inhibiting Ii chain cleavage in mature DCs or cystatin C-negative cells using cat S inhibitors or cystatin C transfection restores a lysosomal pathway. This strategy is used by at least one parasitic nematode which releases a cystatin C homolog to negatively regulate the MHC class II pathway and possibly evade immune detection (Manoury et al., 2001).

Even more interesting is the fate of MHC II that has accumulated in lysosomes prior to maturation. These molecules are somewhat more susceptible to degradation than those on the cell surface (Cella et al., 1997; Pierre et al., 1997), but nevertheless survive 1–2 days. Using an antibody to a hen egg lysozyme (HEL) peptide-MHC II (I-A^k) complex, maturation has been found to enhance the formation of peptide-loaded complexes within the confines of lysosomes (Inaba et al., 2000). These complexes then appear in CIIVs, seemingly being sorted away from resident lysosomal components including undegraded HEL (Turley et al., 2000). The CIIVs may deliver the complexes to the surface (Figure 1, right). This pathway of lysosome to plasma membrane vesicle transport might be a DC-specific specialization, or at least a pathway that is amplified or synchronously activated in maturing DCs. Moreover, these peptide-MHC complexes may reach the cell surface as small clusters, partly associated with CD86 (Turley et al., 2000). This specialization may contribute to the efficiency at which DCs display components of the immunological synapse.

The entire lysosomal system in DCs appears distinguished from that in other cells by its ability to attenuate its proteolytic potential. Whereas cells such as macrophages are adept at rapidly degrading internalized proteins to amino acids, DC lysosomes can sequester antigen for extended periods and still efficiently use the antigen for forming peptide-MHC class II complexes (Turley et al., 2000). We believe DC lysosomes provide a developmentally regulated environment adapted for the formation of T cell receptor ligands. How this regulation occurs is unclear, but may reflect overall alterations in lysosomal enzyme content or lysosomal pH. The immunosuppressive cytokine IL-10 may act in this way to restrict the proteolytic function of monocyte-derived DCs (Fiebiger et al., 2001).

Antigen Presentation on MHC Class I: Breaking Convention

Compared to MHC class II, relatively little effort has yet been focused on MHC class I-restricted antigen presentation in DCs. MHC class I does not accumulate in the lysosomes of immature mouse DCs (although it may in immature LCs), but it too is upregulated upon maturation, possibly reaching the surface in part together with class II molecules (Turley et al., 2000). Maturation may drive the formation of "immunoproteasomes," a combinatorial form of the proteasome that influences the peptides destined to be presented on MHC class I. Most intriguingly, DCs are specialized to form MHC class I peptide complexes by what is called the "exogenous" or "cross presenting" pathway.

Classically, the MHC I pathway provides for the presentation of endogenous cellular antigens. For example, in virus-infected cells, viral proteins expressed in the cytosol are subject to proteasomal proteolysis and the resulting peptides translocated via TAP transporters into the ER lumen for loading onto waiting MHC I molecules. This paradigm may provide some difficulty for DCs, however. A priori, it seems unlikely that all viruses can infect DCs. One alternative is that DCs encounter infected cells in the periphery, phagocytosing the dying cells via receptors specific for such forms of uptake (e.g., α_V β_5 integrins). Indeed, immature DCs take up dying cells avidly and then "cross-present" viral antigens from the infected cells to $CD8^+$ T cells (Albert et al., 1998). Several reports have shown that DCs also process tumor antigens from phagocytosed tumor cells for presentation on MHC I (and MHC II).

Soluble exogenous antigens also can be cross-presented from nonreplicating microbes and immune complexes, the latter taken up via Fc receptors (Rodriguez et al., 1999). Uptake of the same antigens by FcγR on macrophages and B cells does not result in efficient MHC I-restricted presentation. DCs seem to allow the egress of the internalized antigen (3–12 kDa fragments) from endocytic organelles into the cytosol (Rodriguez et al., 1999). From here, the fragments enter the "conventional" MHC I pathway involving proteasomal proteolysis and TAP translocation. Exogenous pathways to MHC class I are especially evident in DCs, but other cells, like liver sinusoidal endothelial lining cells and macrophages, may have this potential. Although little is known about the mechanism of cross-presentation, we suspect far more will rapidly emerge concerning the cell biology of this pathway and its regulation during DC maturation.

Antigen Presentation and Tolerance

Transferring what is being learned in vitro to DCs in situ represents an important challenge. One approach to the problem will involve the use of antigens modified to allow selective targeting DC-specific surface receptors. One such receptor is the multilectin DEC-205 (Mahnke et al., 2000). In or preliminary results, test antigens such as HEL were fused to anti-DEC-205 antibodies, injected into mice, and shown to generate DCs with MHC class II-peptide complexes capable of eliciting T cell responses in vitro and in vivo. Interestingly, the in vivo response was transient, rendering the mice refractory to subsequent injections of HEL, even in Freund's adjuvant. In other words, the animals were apparently tolerized to HEL. Tolerance was converted to immunity, however, if the anti-DEC/HEL fusion

protein was injected together with a maturation signal, e.g., anti-CD40. Thus, as predicted from the in vitro studies, immunization requires DC maturation, but tolerance may be induced by DCs that have not been induced to mature. How can this happen if immature cells are incapable of forming MHC-peptide complexes?

So-called immature DCs in lymphoid tissues seem to have one major difference from their in vitro-generated counterparts: the presence of large amounts of MHC products and MHC-peptide complexes on the surface of DCs in vivo (Inaba et al., 1998). While this is a feature of mature DCs, these cells in vivo otherwise exhibit features of immature DCs. Thus, "immature" DCs in lymphoid organs are endocytically active and express relatively low levels of key costimulatory molecules like CD86 and CD40. The capacity to form MHC-peptide complexes in the absence of a maturation stimulus and in the absence of other changes (costimulatory molecules, chemokine receptors) that allow mature DCs to stimulate immunity, may be critical for DCs in situ to be able to tolerize T cells in the steady state.

In constructing a cell type with a broad and efficient capacity for antigen presentation (Figure 2), the immune system seems to have engaged in a Faustian bargain. The factors that contribute to the DC's capacity for immunity create an equivalent potential for the generation of T cell responses to self antigens and environmental proteins. In fact, DCs in intestinal lymphatics constitutively seem to have captured proteins placed in the gut lumen and fragments of epithelial cells themselves (Huang et al., 2000). Likewise, DCs that line our airways transport soluble macromolecules from the airway to the lymph nodes in the chest (Vermaelen et al., 2001). In these cases, if DCs were to be immunogenic, chronic inflammation would ensue. However, there is growing evidence that DCs in lymphoid organs maintain peripheral tolerance (Heath and Carbone, 2001).

We regard the role of DCs in peripheral tolerance to be intimately related to their function in immunity, the latter depending on DC maturation signals. Many self-reactive T cells must escape the negative selection events in the thymus. Other self antigens may never reach the thymus, particularly during neonatal life when the T cell repertoire begins to be shaped. Similarly, many environmental proteins, e.g., microbial and plant proteins, bathe our respiratory tract and intestines beginning postnatally, long after the thymus has had a chance to delete T cells that are potentially reactive to "self." When an inflammatory or infectious situation arises, the maturing DCs are likely to present at least some of these self and environmental proteins together with the microbial peptides. Some of the specializations in Figure 2 would allow DCs to present these proteins on MHC class I and II in the steady state, prior to encounter with pathogens. As such, the capacity of immature DCs to delete antigen-specific T cells peripherally, or to generate regulatory T cells, may safeguard against autoimmunity and chronic inflammation when infection strikes.

At first, the processing of proteins from other cells seems to violate the beauty of MHC restriction. This elegant part of cell-mediated immunity focuses T cells on infected targets. Cross presentation, in effect, moves peptides from infected cells to noninfected DCs. Because it occurs primarily in DCs, however, cross presentation is, we suspect, critical in tolerizing the T cell repertoire peripherally, and also allows simultaneous sampling of both the MHC class I and class II systems. MHC restriction therefore remains intact for other cells and stages of the T cell response.

The analysis of DC function in T cell-mediated immunity has always involved three parallel paths of research: the uptake and presentation of antigens, the cytokines and surface molecules that control the quality and quantity of the T cell response, and the properties required to distribute and mobilize DCs in vivo. We have considered mechanisms underlying the first topic, stressing the specializations of these antigen processing machines and the importance of DC maturation (Figure 2). A major unknown remains the endocytic and processing capacities of DCs in situ. The cell biology of DCs in vivo will benefit considerably from approaches that involve selective targeting of antigens to DCs in situ, as in the DEC-205 work cited above, imaging studies, selective genetic manipulation of DCs in situ, and continued attention to fundamental mechanisms defined in culture. Understanding the cell biological basis for DC function has emerged as an important and rich area. We suspect it will hold the key to understanding how DCs maintain the balance between tolerance for self and immunity against pathogens.

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